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Chromosome pairing in durum wheat haploids with and without *ph1b* of bread wheat

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Abstract Durum or macaroni wheat (*Triticum turgidum* L., $2n = 4x = 28$; AABB) is an allotetraploid with two related genomes, AA and BB, each with seven pairs of homologous chromosomes. Although the corresponding chromosomes of the two genomes are potentially capable of pairing with one another, the *Ph1* (*Pairing homoeologous*) gene in the long arm of chromosome 5B permits pairing only between homologous partners. As a result of this *Ph1*-exercised disciplinary control, durum wheat and its successor, bread wheat (*Triticum aestivum* L., $2n = 6x = 42$; AABBDD) show diploid-like chromosome pairing and hence disomic inheritance. The *Ph* mutants in the form of deletions are available in bread wheat (*ph1b*) and durum wheat (*ph1c*). Thus, *ph1b*-haploids of bread wheat and *ph1c*-haploids of durum wheat show extensive homoeologous pairing that has been shown by us and several others. Here we study the effect of *ph1b* allele of bread wheat on chromosome pairing in durum haploids, whereas we studied earlier the effect of *ph1c* allele in durum haploids that we synthesized. In durum wheat, the *ph1b*-haploids show much higher (49.4% of complement) pairing than the *ph1c*-haploids (38.6% of complement).

Keywords Fluorescent genomic in situ hybridization (fl-GISH) · Homoeologous chromosome pairing · *Ph1* · *ph1b* · Regulation of chromosome pairing

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Introduction

Durum or macaroni wheat (*Triticum turgidum* L., $2n = 4x = 28$; AABB) is an allotetraploid with two related genomes, AA and BB. Although the corresponding (homoeologous) chromosomes of these genomes are genetically and evolutionarily related and hence potentially capable of pairing with one another, pairing occurs only between homologous partners resulting in strictly diploid-like pairing and disomic inheritance. Bread wheat (*Triticum aestivum* L., $2n = 6x = 42$; AABBDD), which resulted from tetraploid wheat after gaining the DD genome, is also a disomic polyploid. Regular, diploid-like pairing in these polyploid wheats is ensured by the activity of a gene, *Ph1* (*Pairing homoeologous*), in the long arm of the chromosome 5B (Riley and Chapman 1958; Sears

and Okamoto 1958). Such a *Ph1*-enforced pairing was essential for the evolution of these wheats (see Jauhar 2003a). Cytogenetic manipulation of this pairing is also of great interest from the standpoint of alien gene transfer into wheat. Ways and means of inducing homoeologous pairing need to be explored.

Because *Ph1* is hemizygous-effective, its single dose is fully functional in suppressing homoeologous pairing in polyhaploids of both bread wheat (Jauhar et al. 1991) and durum wheat (Jauhar et al. 1999). Thus, polyhaploids with and without *Ph1* constitute excellent tools for studying the amount of chromosome pairing (Jauhar and Joppa 1996; Luo et al. 1996). These haploids also allow the study of secondary associations—side-by-side (s–s), end-to-end (e–e), and end-to-side (e–s)—(Person 1955) between intergenomic univalents, although the significance of such associations has been controversial. A high-pairing mutation, designated *ph1b* (Sears 1977), involving deletion of *Ph1* in 5BL, induces substantial intergenomic chromosome pairing in bread wheat, especially in its haploids (Jauhar et al. 1991). A similar mutation, called *ph1c*, was induced in durum wheat (Giorgi 1978). Durum haploids with *ph1c* also show considerable homoeologous pairing (Jauhar et al. 1999). The *Ph1*-controlled pairing is clearly relaxed in the *ph1b* and *ph1c* mutants, making it possible to study and compare the degree of chromosome pairing in haploids with these alleles.

Earlier, we studied chromosome pairing in *ph1b*-haploids of bread wheat (Jauhar et al. 1991) and *ph1c*-haploids of durum wheat (Jauhar et al. 1999). We used the maize method (Jauhar 2003b) to extract haploids from the heterozygous stock of the durum cultivar Langdon, LDN *Ph1/ph1b* and obtained, through segregation in egg cells, Langdon haploids with *Ph1* and with *ph1b* in the same genetic background. In this paper, we report on chromosome pairing in durum haploids with and without *ph1b* allele of bread wheat and also compare it with chromosome pairing in *ph1c*-haploids of durum.

Materials and methods

A durum wheat genetic stock J93—Langdon *Ph1/ph1b*—provided by Leonard Joppa of the Cereals Group, USDA–ARS, Fargo, North Dakota, was used for extracting polyhaploids by the maize method. This stock called, LDN *Ph1/ph1b*, has the pedigree: Chinese Spring (*ph1b/ph1b*)/2*Langdon//2*durum line 47-1. It was obtained by crossing Chinese Spring *ph1b/ph1b* mutant with Langdon, backcrossing to Langdon, followed by a cross to durum line 47-1 (a rust-susceptible Langdon material, registered as 'Rusty' Klindworth et al. 2006), and then a second backcross to durum Rusty.

LDN *Ph1/ph1b* was crossed with maize using the methods of emasculation, pollination, and post-pollination treatments we standardized earlier (Almouslem et al. 1998; Jauhar 2003b). The embryo-derived plantlets were first grown in the growth chamber. During developmental stages of pre- or post-rescue embryos (initially with 24 somatic chromosomes, 14 of durum and 10 of maize parent), the 10 somatic chromosomes of maize are eliminated resulting in durum haploids with 14 chromosomes. Somatic chromosome counts were used to verify the haploid status of the plantlets which were later transferred to the greenhouse. Because of segregation in egg cells, the heterozygous line LDN *Ph1/ph1b* produced two types of haploids, with *Ph1* or *ph1b* allele.

Chromosome pairing was studied at meiotic metaphase in suitably stained PMCs according to techniques described previously (Jauhar et al. 1999; Jauhar 2003b). We also employed fluorescent genomic in situ hybridization (fl-GISH) to discriminate between the A- and B-genome chromosomes and verify the euploid status of the plantlets. Secondary associations—side-to-side (s–s), end-to-end (e–e), and end-to-side (e–s)—between intergenomic univalents were also analyzed. Fl-GISH was conducted by hybridizing the A-genome with the *Triticum urartu* Tum. genomic DNA labeled with biotin-14-dATP in the amount of 100 ng/slide. The B genome was blocked with *Aegilops speltoides* Tausch genomic DNA (2,000 ng/slide), essentially according to Jauhar et al. (1999). Propidium iodide (PI) was used to counterstain the chromosome preparations and the labeled DNA was detected using fluorescein isothiocyanate (FITC).

Results and discussion

Haploid plants have been fruitfully employed in cytogenetic studies, especially on genomic and phylogenetic relationships (Maluszynski et al. 2003; Ceoloni

and Jauhar 2006). They offer an excellent opportunity of studying chromosome pairing because the internal homologies that remain masked in the disomic state are revealed in the haploid complement with only a single dose of each chromosome. Although the effect of the absence of *Ph1* is often studied in F_1 hybrids between wheat and a related species like rye (*Secale cereale* L.), the wheat haploids offer a better material for such studies because there is no alien genome to influence pairing. The absence of *Ph1* substantially promotes homoeologous chromosome pairing in wheat polyhaploids. Chromosome pairing has been studied in *ph1b*-haploids of bread wheat (Jauhar et al. 1991), *ph1c*-haploids of durum wheat (Jauhar et al. 1999), and in the durum 5D(5B) substitution haploids (Doğramacı-Altuntepe and Jauhar 2001). Now we have studied chromosome pairing in durum haploids with and without the *ph1b* allele of bread wheat.

Because the absence of *Ph1* induces homoeologous, and sometimes non-homologous, pairing, segmental interchanges and karyotypic alterations (Sánches-Morán et al. 2001) would be expected in haploids derived from *ph1b/ph1b* or *ph1c/ph1c* mutant lines. Such alterations could cause a bias in chromosome pairing studies. Since the heterozygote LDN *Ph1/ph1b* has no homoeolgous pairing because of the presence of dominant *Ph1*, the haploids obtained from it would not be expected to have any karyotypic alterations. Therefore, we extracted haploids from this heterozygous line by crossing it with maize.

A total of seven durum haploids ($2n = 2x = 14$) were obtained from the LDN *Ph1/ph1b* stock by crossing it with maize. Of these haploids, five were studied with regard to chromosome pairing (Table 1). Because of segregation in gametes (egg cells) produced by the heterozygous parental line, durum haploids with *Ph1* or *ph1b* allele were obtained after crossing with maize. Three of the haploids, HP-45, HP-66, and HP-67, showed very little chromosome pairing, if any, (Fig. 1A, B) and were obviously *Ph1*-haploids. Fl-GISH analysis on meiotic chromosomes confirmed the euploid status of these plants as they showed seven A-genome and seven B-genome univalents (Fig. 2A, B). These *Ph1*-haploids also afforded the study of secondary associations—side-to-side (s–s), end-to-end (e–e), and end-to-side (e–s) (Person 1955; Jauhar 1970)—between intergenomic univalents. We analyzed such associations in 63 probed PMCs and observed associations as follows:

Within the A genome: 4 s–s and 1 e–e
Within the B genome: 3 s–s and 1 e–s
Between the A and B genomes: 19 s–s, 1 e–e, and 1 e–s

Although the cytogenetic or phylogenetic significance of secondary associations is not well recognized (Sadasivaiah 1974), it is interesting that intergenomic s–s associations (Fig. 2A), which reflect genomic closeness (Person 1955; Jauhar 1970), were observed in a much larger proportion of PMCs than were the intragenomic s–s associations.

Table 1 Chromosome pairing in durum haploids ($2n = 2x = 14$; AB) with and without *ph1b* of bread wheat

Haploid	No. of PMCs	Mean and range of chromosome configurations at metaphase I								Chiasma frequency per cell	Percent complement paired
		III		Ring II		Rod II		I			
		Mean	Range	Mean	Range	Mean	Range	Mean	Range		
With *Ph1*											
HP-45	50	–	–	–	–	0.24	(0–1)	13.52	(12–14)	0.24	3.4
HP-66	50	–	–	–	–	0.16	(0–1)	13.68	(12–14)	0.16	2.3
HP-67	50	–	–	–	–	0.28	(0–2)	13.72	(10–14)	0.28	4.0
Overall Mean		–	–	–	–	0.23		13.64		0.23	3.2
With *ph1b*											
HP-8	100	0.11	(0–1)	0.36	(0–3)	2.76	(0–5)	7.43	(1–14)	3.73	46.9
HP-8b	50	0.20	(0–1)	0.48	(0–3)	2.84	(0–5)	6.36	(1–14)	4.20	51.7
Overall Mean		0.16		0.42		2.80		7.05		3.97	49.4

Abbreviations: PMC, pollen mother cell; III, trivalent; II, bivalent; I, univalent

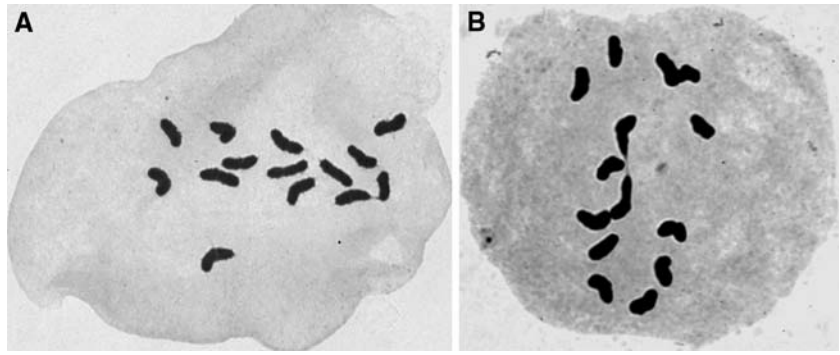

Fig. 1 Chromosome pairing in conventionally stained PMCs of durum haploids derived from Langdon *Ph1/ph1b* by crossing with maize. Haploids with *Ph1* (**A**, **B**) show no or very little homoeologous pairing. (**A**) PMC with 14 univalents; note complete lack of pairing because of the presence of *Ph1*. (**B**) PMC with one rod bivalent (II) plus 12 univalents (I)

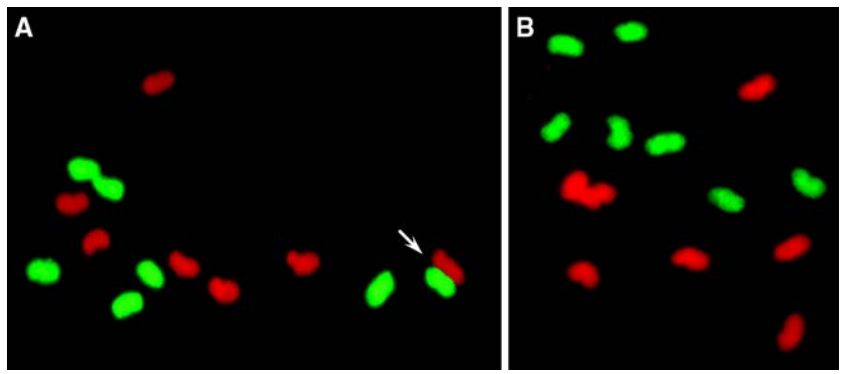

Fig. 2 Fluorescent genomic in situ hybridization (fl-GISH) analysis of meiotic chromosomes of *Ph1b*-haploids derived from Langdon *Ph1/ph1b* by crossing with maize. The A genome was probed with *Triticum urartu* genomic DNA labeled with biotin and detected with fluorescein isothiocyanate (FITC); the B genome was blocked with *Aegilops speltoides* DNA and revealed using propidium iodide (PI) counterstain. **A** , **B.** PMCs showing 14 univalents, 7 I from the A genome (green) and 7 I from the B genome (red). Note complete absence of pairing between the A-genome and the B-genome chromosomes; intergenomic chromosome association (side-to-side) is noticeable (arrow)

Two of the haploids, HP-8 and HP-8b, were *ph1b*-haploids and had substantial pairing (Fig. 3A–D). Trivalents and ring bivalents were observed in addition to rod bivalents (Fig. 3C). In the presence of *Ph1*, on an average 3.2% of the haploid chromosome complement paired with 0.23 chiasma per cell; in contrast, 49.4% pairing and 3.97 chiasmata per cell were observed in the *ph1b*-haploids of durum wheat (Table 1), an almost 15-fold increase in pairing. The proportion of chromosome pairing in these *ph1b*-haploids is comparable to that in haploids lacking the entire chromosome 5B, as shown in nulli-5B haploids of bread wheat with 54.9% pairing (Riley and Chapman 1958) or in substitution 5D(5B) haploids of durum wheat with 51.3% pairing (Jauhar et al. 1999). Martinez et al. (2005) observed differences in chromosome pairing in maize-derived *Ph1*-haploids of three bread wheat cultivars, Thatcher, Chris, and Chinese Spring. Thatcher and Chris haploids had significantly higher level of pairing than Chinese Spring.

Although differences in the effectiveness of *Ph1* in suppressing homoeologous pairing in haploids of different cultivars are known (Jauhar et al. 1991; Martinez et al. 2005), it is difficult to explain higher chromosome pairing (49.4%) in durum haploids with the *ph1b* allele of bread wheat, compared to 38.57% in durum haploids with the *ph1c* allele of durum wheat (Jauhar et al. 1999). Both of these mutations involve interstitial deletions, the *ph1b* mutation involving a deletion of 1.05 μm (about 70 Mb) (Gill et al. 1993, 1996), whereas the *ph1c* mutation involves a deletion of 0.89 μm (Gill et al. 1993). The *Ph1* is believed to

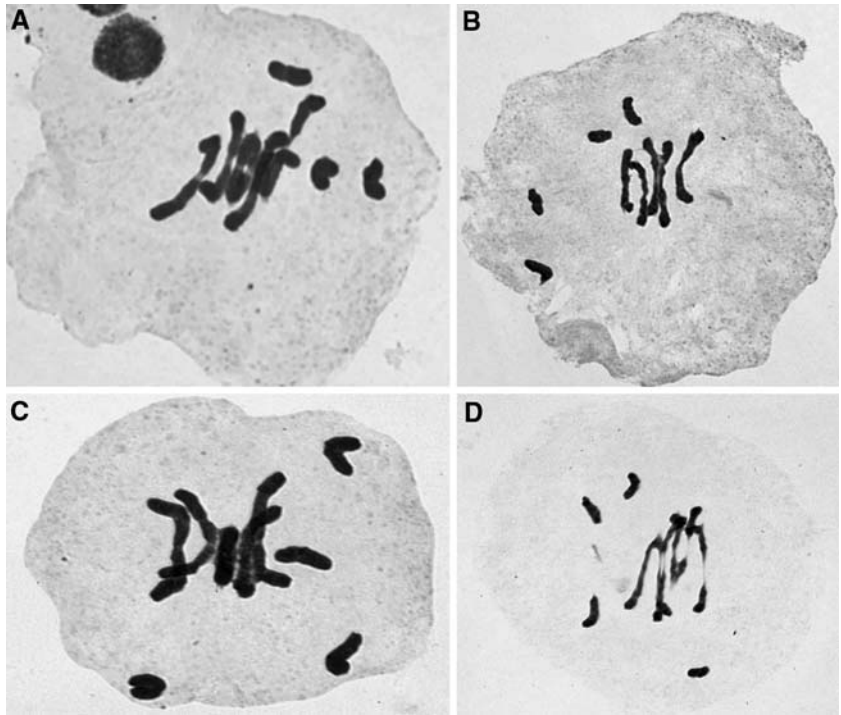

Fig. 3 Chromosome pairing in conventionally stained PMCs of durum haploids derived from Langdon *Ph1/ph1b* by crossing with maize. Extensive homoeologous pairing is present in PMCs of two *ph1b*-haploid plants, HP-8 (**A**–**C**) and HP-8b (**D**). (**A**) One trivalent (III), 1 ring II, 3 rod II, and 3 I; note high pairing in the presence of *ph1b*. (**B**) Five rod II plus 4 I. (**C**) Four rod II plus 6 I. (**D**) Two III, 2 rod II, and 4 I

be a complex locus (Griffiths et al. 2006) that affects premeiotic association of homologous chromosomes and timing of their intimate association (Martinez-Perez et al. 1999), and recombination among homoeologous chromosomes (Dubcovsky et al. 1995). It may be surmised that the absence of *Ph1* in the larger *ph1b* deletion could perhaps cause higher homoeolgous pairing in the *ph1b*-haploids than in the smaller deletion in the *ph1c*-haploids of durum wheat.

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